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(54) Title: GALANIN RECEPTOR GALR2 AND NUCLEOTIDES ENCODING SAME

(57) Abstract

A new galanin receptor, GALR2, is described. Also provided are nucleic acids encoding same and various assays to identify ligands particular to said receptor. Ligands so identified are useful for the treatment of obesity, treatment of pain, and treatment of cognitive disorders.

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TITLE OF THE INVENTION

GALANIN RECEPTOR GALR2 AND NUCLEOTIDES ENCODING SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

Not applicable

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not applicable

REFERENCE TO MICROFICHE APPENDIX

Not applicable

FIELD OF THE INVENTION

This invention relates to a novel galanin receptor, designated GALR2, to nucleotides encoding it, and to assays which use it.

BACKGROUND OF THE INVENTION

Although first isolated from porcine intestine, galanin is widely distributed in the central and peripheral nervous system. Galanin in most species is a 29 amino acid peptide with an amidated carboxyl terminus. Human galanin is unique in that it is longer, 30 amino acids, and is not amidated. There is strong conservation of the galanin sequence with the amino terminal fifteen residues being absolutely conserved in all species. Galanin immunoreactivity and binding is abundant in the hypothalamus, the locus coeruleus, the hippocampus and the anterior pituitary, as well as regions of the spinal cord, the pancreas and the gastrointestinal tract.

Like neuropeptide Y (NPY), injection of galanin into the paraventricular nucleus (PVN) of the hypothalamus produces a dose-dependent increase in feeding in satiated rats. While galanin, like norepinephrine, enhances carbohydrate ingestion, some studies have shown that it profoundly increases fat intake. It has been suggested that

galanin shifts macronutrient preference from carbohydrate to fat. The same injections that increase feeding reduce energy expenditure and inhibit insulin secretion. There is enhanced galanin expression in the hypothalamus of genetically obese rats compared with their lean littermate controls. Injection of peptide receptor antagonists into the PVN blocks the galanin-specific induction of increased fat intake. Specific galanin antisense oligonucleotides when injected into the PVN produce a specific decrease in galanin expression associated with a decrease in fat ingestion and total caloric intake while hardly affecting either protein or carbohydrate intake. Thus galanin appears to be one potential neurochemical marker related to the behavior of fat ingestion.

Galanin inhibits cholinergic function and impairs working memory in rats. Lesions that destroy cholinergic neurons result in deficits in spatial learning tasks. While locally administered acetylcholine (ACh) reverses some of this deficit, galanin blocks this ACh-mediated improvement. Evidence from autopsy samples from Alzheimer's disease-afflicted brains suggests an increased galinergic innervation of the nucleus basilis. Thus, if galinergic overactivity contributes to the decline in cognitive performance in Alzheimer's disease, galanin antagonists may be therapeutically useful in alleviating cognitive impairment.

In the rat, administration of galanin intracerebroventricularly, subcutaneously or intravenously increases plasma growth hormone. Infusion of human galanin into healthy subjects also increases plasma growth hormone and potently enhances the growth hormone response to GHRH.

Galanin levels are particularly high in dorsal root ganglia. Sciatic nerve resection dramatically up-regulates galanin peptide and mRNA levels. Chronic administration of galanin receptor antagonists (M35, M15) after axotomy results in a marked increase in self mutilation behavior in rats, generally considered to be a response to pain. Application of antisense oligonucleotides specific for galanin to the proximal end of a transected sciatic nerve suppressed the increase in galanin peptide levels with a parallel increase in autotomy. Galanin injected intrathecally acts synergistically with morphine to produce analgesia, this antinociceptive effect of morphine is blocked by galanin

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receptor antagonists. Thus, galanin agonists may have some utility in relieving neural pain.

The actions of galanin are mediated by high affinity galanin receptors that are coupled by pertussis toxin sensitive G_i/G_o proteins to inhibition of adenylate cyclase activity, closure of L-type Ca^{++} channels and opening of ATP-sensitive K^+ channels. Specific binding of ^{125}I-galanin (Kd approximately 1 nM) has been demonstrated in areas paralleling localization of galanin immunoreactivity: hypothalamus, ventral hippocampus, basal forebrain, spinal cord, pancreas and pituitary. In most tissues the amino terminus (GAL 1-15) is sufficient for high affinity binding and agonist activity.

Recently, a galanin receptor cDNA was isolated by expression cloning from a human Bowes melanoma cell line. (Habert-Ortoli, et al. 1994. *Proc. Nat. Acad. Sci,, USA* 91: 9780-9783). This receptor, GALR1, is expressed in human fetal brain and small intestine, but little else is known of its distribution. Gal(1-16) is at least 1000 times more active than pGAL(3-29) as an inhibitor of ^{125}I-porcine galanin binding to this receptor transiently expressed in COS cells. It remains to be determined whether this receptor subtype represents the hypothalamic receptor that mediates the galanin specific feeding behavior.

It would be desirable to identify further galanin receptors so that they can be used to further characterize this biological system and to identify galanin receptor subtype selective agonists and antagonists.

SUMMARY OF THE INVENTION

This invention relates to a novel galanin receptor, designated GALR2, substantially free from associated proteins, and to GALR2-like receptors which are at least about 40% homologous and which have substantially the same biological activity. In preferred embodiments of this invention, the GALR2-like receptors are at least about 60%, and more preferably at least about 75%, and even more preferably at least about 85% homologous to a GALR2 receptor. This invention also relates specifically to rat, human and mouse GALR2, substantially free from associated proteins, and to receptors which are at

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least about 50% homologous and which have substantially the same biological activity.

Another aspect of this invention are primate and non-primate GALR2 proteins which are encoded by substantially the same nucleic acid sequences, but which have undergone changes in splicing or other RNA processing-derived modifications or mutagenesis-induced changes, so that the expressed protein has a homologous, but different amino acid sequence from the native forms. These variant forms may have different and/or additional functions in human and animal physiology or *in vitro* in cell based assays.

A further aspect of this invention are nucleic acids which encode a galanin receptor or a functional equivalent from rat, human, mouse, swine, or other species. These nucleic acids may be free from associated nucleic acids, or they may be isolated or purified. The nucleic acids which encode a receptor of this invention may be any type of nucleic acid. Preferred forms are DNAs, including genomic and cDNA, although this invention specifically includes RNAs as well. Nucleic acid constructs may also contain regions which control transcription and translation such as one or more promoter regions, termination regions, and if desired enhancer regions. The nucleic acids may be inserted into any known vector including plasmids, and used to transfect suitable host cells using techniques generally available to one of ordinary skill in the art.

Another aspect of this invention are vectors comprising nucleic acids which encode GALR2, and host cells which contain these vectors. Still another aspect of this invention is a method of making GALR2 comprising introducing a vector comprising nucleic acids encoding GALR2 into a host cell under culturing conditions.

Yet another aspect of this invention are assays for GALR2 ligands which utilize the receptors and/or nucleic acids of this invention. Preferred assays of this embodiment compare the binding of the putative GALR2 ligand to the binding of galanin to GALR2.

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BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1. is the nucleic acid sequence of rat GALR2 (clone 27A) containing 5' and 3' untranslated regions (SEQ ID NO:1).

FIGURE 2 is the nucleic acid sequence of GALR2 (clone 27A) from initiator Met to termination codon (SEQ ID NO: 2).

FIGURE 3 is a schematic representation of GALR2 (clone 27A) and the nucleic acid and deduced amino acid sequence of GALR2 (clone 27A) (SEQ ID NOS: 3 and 4).

FIGURE 4 is the deduced amino acid sequence of GALR2 (clone 27A) (SEQ ID NO: 5).

FIGURE 5 is a comparison (PileUp alignment) of amino acid sequences for rat GALR1 (SEQ ID NO: 6) and rat GALR2 (SEQ ID NO:7).

FIGURE 6 is the nucleic acid sequence of the cDNA probe used to isolate GALR2 (SEQ ID NO:8).

FIGURE 7 is the DNA sequence of human GALR2 gene (SEQ ID NO:9).

FIGURE 8 is the DNA sequence (open reading frame only) of human GALR2 gene (SEQ ID NO:10).

FIGURE 9 is the deduced amino acid sequence of human GALR2 (SEQ ID NO:11).

FIGURE 10 demonstrates the pharmacology of human and rat GALR2.

FIGURE 11 illustrates G_q or G_s coupled response (pigment dispersion) as well as G_i-coupled response (pigment aggregation).

FIGURE 12 is the DNA sequence of mouse GALR2 gene (SEQ ID NO:12).

FIGURE 13 is the amino acid sequence for mouse GALR2 gene (SEQ ID NO:13).

FIGURE 14 is a comparison of human, rat and mouse GALR1 and GALR2 protein sequences showing strong sequence conservation amoung members of the GALR gene family.

FIGURE 15 is the RNA expression profile of human GALR2.

FIGURE 16 illustrates the expression of rat GALR2 in the brain.

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DETAILED DESCRIPTION OF THE INVENTION

As used throughout the specification and claims, the following definitions apply:

"Substantially free from associated proteins" means that the receptor is at least about 90%, and preferably at least about 95% free from other cell membrane proteins which are normally found in a living mammalian cell which expresses a galanin receptor.

"Substantially free from associated nucleic acids" means that the nucleic acid is at least about 90%, and preferably at least about 95%, free from other nucleic acids which are normally found in a living mammalian cell which naturally expresses a galanin receptor gene.

"Substantially the same biological activity" means that the receptor-galanin binding constant is within 5-fold of the binding constant of GALR2 and galanin, and preferably within 2-fold of the binding constant of GALR2 and galanin.

"Stringent post-hybridizational washing conditions" means 0.1 X standard saline citrate (SSC) at 65°C.

"Standard post-hybridizational washing conditions" means 6 x SSC at 55°C.

"Relaxed post-hybridizational washing conditions" means 6 x SSC at 30°C, or 1 to 2 X SSC at 55°C.

"Functional equivalent" means that a receptor which does not have the exact same amino acid sequence of a naturally occurring GALR2 protein due to alternative splicing, deletions, mutations, or additions, but retains at least 1%, preferably 10%, and more preferably 25% of the biological activity of the naturally occurring receptor. Such derivatives will have a significant homology with a natural GALR2 and can be detected by reduced stringency hybridization with a DNA sequence obtained from a GALR2. The nucleic acid encoding a functional equivalent has at least about 60% homology at the nucleotide level to a naturally occurring receptor nucleic acid.

It has been found, in accordance with this invention, that there is a second galanin receptor, which is designated GALR2. The rat, human and mouse GALR2 sequences are given in FIGURES 4, 9 and 13, respectively, and are referenced in the Examples; however it is to

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be understood that this invention specifically includes GALR2 without regard to the species and, in particular, specifically includes rodent (including rat and mouse), rhesus, swine, chicken, cow and human. The galanin 2 receptors are highly conserved throughout species, and one of ordinary skill in the art, given the rat, human and/or mouse sequences presented herein, can easily design probes to obtain the GALR2 from other species.

GALR2 proteins contain various functional domains, including one or more domains which anchor the receptor in the cell membrane, and at least one ligand binding domain. As with many receptor proteins, it is possible to modify many of the amino acids, particularly those which are not found in the ligand binding domain, and still retain at least a percentage of the biological activity of the original receptor. Thus this invention specifically includes modified functionally equivalent GALR2s which have deleted, truncated, or mutated N-terminal portions. This invention also specifically includes modified functionally equivalent GALR2s which contain modifications and/or deletions in other domains, which are not accompanied by a loss of functional activity.

Additionally, it is possible to modify other functional domains such as those that interact with second messenger effector systems, by altering binding specificity and/or selectivity. Such functionally equivalent mutant receptors are also within the scope of this invention.

The proteins of this invention were found to have structural features which are typical of the 7-transmembrane domain (TM) containing G-protein linked receptor superfamily (GPC-R's or 7-TM receptors). Thus GALR2 proteins make up new members of the GPC-R family of receptors. The intact GALR2 of this invention was found to have the general features of GPC-R's, including seven transmembrane regions, three intra- and extracellular loops, and the GPC-R protein signature sequence. The TM domains and GPC-R protein signature sequence are noted in the protein sequences of the GALR2. Not all regions are required for functioning, and therefore this invention also comprises functional receptors which lack one or more non-essential domains.

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Determination of the nucleotide sequence indicated that the GALR2 belongs to the intron-containing class of GPC-R's. Clone 27A, a precursor mRNA terminating in a poly (A) tract, encodes a 1119 bp open reading frame divided into two exons by a single intron of approximately 500 bp (FIGURE 4). Exon 1 encodes the N-terminal extracellular domain through predicted TM-3, while exon 2 encodes the second predicted extracellular loop through the C-terminal intracellular domain. A perfectly conserved splice donor site (G/gt) is found at nucleotide 368 which coincides with the second residue of the G protein-coupled receptor signature aromatic triplet, (D,E) RY.

Removal of the intron indicates that clone 27A encodes a full-length rat galanin receptor polypeptide of 372-amino acids with 7 predicted TM domains, as underlined in FIGURE 4. Searches of nucleic acid and protein sequence databases revealed that the open reading frame sequence is unique and most closely related to rat galanin 1 receptor (GALR1) with 55% nucleic acid and 38% protein sequence identity. An alignment of the protein sequences for rat GALR1 and GALR2 is given in FIGURE 5. Several conserved features ascribed to GPC-R's were also identified in the rat GALR2: the signature aromatic triplet sequence (Glu-Arg-Tyr) adjacent to TM-3, Cys-98 and Cys-153 in the first two extracellular loops capable of disulfide bonding, putative amino-terminal N-glycosylation sites (Asn-Xaa-Ser/Thr), phosphorylation sites in the carboxyl-terminus and the third cytoplasmic loop, and conserved proline residues in TM-4, 5, 6 and 7.

A second cDNA clone was isolated, termed clone 16.6, which does not contain an intron and is therefore a contiguous cDNA containing the complete open reading frame of GALR2. Like clone 27A, Clone 16.6 contains a 5' untranslated region of approximately 500 bp, a contiguous GALR2 open reading frame encoding 7-TM domains (1119 bp), a 3' untranslated region of about 320 bp, and a poly (A) tract. The open reading frame sequence is identical for clones 27A and 16.6 except for nucleotide 109 of the open reading frame (located in predicted TM-1). Clone 27A contains a T while Clone16.6 contains a C in position 109. Thus, amino acid 37 of the GALR2 protein is phenylalanine in Clone 16.6 and isoleucine in Clone 27A. Both the DNAs of clones 27A and Clone 16.6 form aspects of this invention, as do their respective proteins.

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The human GALR2 protein bears strong sequence identity and similarity to the rat GALR2 ortholog. One notable difference between the human and rat forms is the presence of an additional 15 amino acids in the C-terminal intracellular domain of human GALR2. The mouse protein sequence, as well, bears very strong identity and similarity with the GALR gene family.

This invention also relates to truncated forms of GALR2, particularly those which encompass the extracellular portion of the receptor, but lack the intracellular signaling portion of the receptor, and to nucleic acids encoding these truncated forms. Such truncated receptors are useful in various binding assays. Thus this invention specifically includes modified functionally equivalent GALR2s which have deleted, truncated, or mutated N-terminal portions. This invention also specifically includes modified functionally equivalent GALR2s including receptor chimeras which contain modifications and/or deletions in other domains, which are not accompanied by a loss of functional activity.

Additionally, it is possible to modify other functional domains such as those that interact with second messenger effector systems, by altering binding specificity and/or selectivity. Such functionally equivalent mutant receptors are also within the scope of this invention.

Assays which make up further aspects of this invention include binding assays (competition for ^{125}I-galanin binding), coupling assays (including galanin-mediated inhibition of forskolin-stimulated adenylate cyclase in cells expressing galanin receptors), measurement of galanin-stimulated calcium release in cells expressing galanin receptors (such as aequorin assays), stimulation of inward rectifying potassium channels (GIRK channels, measured by voltage changes) in cells expressing galanin receptors, and measurement of pH changes upon galanin stimulation of cells expressing galanin receptors as measured with a microphysiometer.

Host cells may be cultured under suitable conditions to produce GALR2. An expression vector containing DNA encoding the receptor may be used for expression of receptor in a recombinant host cell. Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to bacteria such as *E. coli*, fungal cells such as yeast,

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mammalian cells including but not limited to cell lines of human, bovine, porcine, monkey and rodent origin, and insect cells including but not limited to *Drosophila* , *Spodoptera*, and silkworm derived cell lines. Cell lines derived from mammalian species which are suitable and which are commercially available include, but are not limited to, L cells L-M(TK$^-$) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171).

The specificity of binding of compounds showing affinity for the receptor is shown by measuring the affinity of the compounds for cells transfected with the cloned receptor or for membranes from these cells. Expression of the cloned receptor and screening for compounds that inhibit the binding of radiolabeled ligand to these cells provides a rational way for rapid selection of compounds with high affinity for the receptor. These compounds identified by the above assays may be agonists or antagonists of the receptor and may be peptides, proteins, or non-proteinaceous organic molecules. Alternatively, functional assays of the receptor may be used to screen for compounds which affect the activity of the receptor. Such functional assays range from *ex vivo* muscle contraction assays to assays which determine second messenger levels in cells expressing the receptor. The second messenger assays include, but are not limited to, assays to measure cyclic AMP or calcium levels or assays to measure adenyl cyclase activity. These compounds identified by the above assays may be agonists, antagonists, suppressors, or inducers of the receptor. The functional activity of these compounds is best assessed by using the receptor either natively expressed in tissues or cloned and exogenously expressed.

Using the assays of this invention, galanin agonists and antagonists may be identified. A galanin agonist is a compound which binds to the GALR2, such as a galanin mimetic, and produces a cellular response which is at least about equivalent to that of galanin, and which may be greater than that of galanin. Such compounds would be useful in situations where galanin insufficiency causes anorexia, or for treatment of pain.

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Also using this embodiment of the assay, galanin antagonists may be identified. A galanin antagonist is a compound which can bind to the GALR2, but produces a lesser response than that of native galanin. Such compounds would be useful in the treatment of obesity.

One assay of this invention is a method of identifying a compound which modulates GALR2 receptor comprising: a) culturing cells expressing the GALR2 receptor in the presence of the compound and b) measuring GALR2 receptor activity or second messenger activity. If desired, the determined activity can be compared to a standard, such as that measured using galanin as the compound. In preferred embodiments, the cells are transformed and express the GALR2 receptor.

The consultant cDNA clone (or shorter portions of, for instance, only 15 nucleotides long) may be used to probe libraries under hybridization conditions to find other receptors which are similar enough so that the nucleic acids can hybridize, and is particularly useful for screening libraries from other species. In this step, one of ordinary skill in the art will appreciate that the hybridization conditions can vary from very stringent to relaxed. Proper temperature, salt concentrations, and buffers are well known.

The following non-limiting Examples are presented to better illustrate the invention.

EXAMPLE 1

A cDNA library from rat hypothalamus was constructed in the plasmid-based mammalian vector pcDNA-3 (InVitrogen, San Diego, CA). Total RNA was isolated from freshly-dissected rat hypothalami (flash-frozen in liquid nitrogen) using the RNagents total RNA isolation kit (Promega Biotech, Madison, WI) with a yield of approximately 0.5 mg from 1 g (wet weight) of hypothalamic tissue. Poly $(A)^+$ mRNA was selected using the Poly A tract mRNA Isolation System III (Promega Biotech) with a yield of approximately 6 µg from 0.5 µg total RNA. 3 µg of poly $(A)^+$ was then utilized as a template for cDNA synthesis using a kit (Choice Superscript, Life Technologies, Gaithersberg, MD) with both

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random hexamer and oligo (dT)-Not I priming. The double-stranded cDNA was adapted for insertion into the BstXI site of pCDNA-3 using EcoRI/BstXI adapters and transformed by electroporation into the *E.coli* strain HB101. The resulting library contained approximately 750,000 primary transformants with 90% of the clones containing inserts (average size 1-2 kb). The library (approximately 700,000 cfu) was plated onto LB plates containing ampicillin and chloramphenicol and probed with a approximately 280 bp PCR fragment (SEQ ID NO:8). Hybridization was conducted at 32°C for 18 hrs. in 5 X SSPE buffer containing 50% formamide, 4 X Denhardt's solution, 0.1% SDS, 10% dextran sulfate, 30 μg/ml sheared salmon-sperm DNA with 2 x 10^6 cpm/ml of ^{32}P-labeled probe. The probe was radiolabeled by random-priming with [α]^{32}P-dCTP to a specific activity of greater than 10^9 dpm/μg. The filters were then washed in 1 x SSC, 0.1% SDS at 55°C and exposed to film (Kodak X-omat) for 48 hrs. Two independent positive clones were identified (clones 27A and 16.6) and subjected to further analysis.

EXAMPLE 2

Sequence Analysis of GALR2

DNA was prepared from overnight cultures using the Wizard DNA Purification System (Promega Corp., Madison, WI) and subjected to automated sequence analysis using the PRISM Dye Deoxy terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) on an ABI 377 instrument. Initial sequencing primers were complementary to the T7 and SP6 promoter sites in pcDNA-3, additional primers were made complementary to the insert DNA. Database searches (Genbank, EMBL, Swiss-Prot, PIR, dEST, Prosite, dbGPCR), sequence alignments, and analysis of the galanin receptor nucleotide and protein sequences were carried out using the GCG Sequence Analysis Software Package (Madison, WI; pileup, peptide structure and motif programs), FASTA and BLAST search programs, and the PC/Gene software suite from Intelligenetics (San Francisco, CA; protein analysis programs).

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EXAMPLE 3

Construction of a Vector for Expression of GALR2

Five μg of the mammalian expression vector pCI.neo (Promega Biotech, Madison WI) was digested with 20 units of EcoRI for 2 hours at 37°C. The digest was then treated with calf intestinal phosphatase and then electrophoresed on 1% Seaplaque gel in 1X TAE buffer and the band corresponding to linearized vector was cut out. DNA was recovered from the slice after melting at 65°C using the Promega Wizard PCR system (Promega Biotech). DNA was quantitated by electrophoresis with standards on a 1% TBE gel. 100 ng of the 2200 bp EcoRI insert (including the intron) from pCDNA-3/27A was ligated to 50 ng of the vector pCI.neo in a 10 ml reaction at room temperature for 1 hour. 1 μl of this ligation mixture was used to transform 50 μl competent DH5a cells (Life Technologies). Clones in the correct orientation were selected following a digest with BamHI. Transfection-quality DNA was then prepared using the Qiagen Maxi protocol (Qiagen, Chatsworth, CA). Mammalian COS-7 cells were transfected by electroporation. COS-7 cells (1×10^7) were suspended in 0.85 ml of Ringers' buffer and 15 mg of the pCI.neo/27A clone was added to a 0.4 mm electroporation cuvette (Bio-Rad, Hercules, CA). Current was applied (960 μF, 260 V) using a Bio-Rad Electroporator device and the cells were transferred to a T-180 flask (Corning). Expression was allowed to proceed for 72 hrs.

EXAMPLE 4

Pharmacology of GALR2

Membranes were prepared from transfected cells following dissociation in enzyme-free dissociation solution (Specialty Media, Lavallette, NJ) by disruption in a Dounce homogenizer in ice-cold membrane buffer (10 mM Tris, pH 7.4, 10 mM PMSF, 10 μM phosphoramidon, and 40 μg/ml bacitracin). After a low speed (1100 x g for 10 min. at 4°C) and a high speed centrifugation (38,700 x g for 15 min.

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at 4°C), membranes were resuspended in buffer and protein concentration determined (Bio-Rad assay kit). Binding of ^{125}I-human galanin (specific activity of 2200 Ci/mmol, DuPont NEN) was measured in membranes using a buffer of 25 mM Tris pH 7.4, 0.5% BSA, 2 mM $MgCl_2$, 40 μg/ml bacitracin, 4μg/ml phosphoramidon, and 10 μM leupeptin in a total volume of 250 μl. 70 pM ^{125}I-human galanin was used. Reactions were initiated by the addition of membranes and the incubation was allowed to proceed at room temperature for 1 hour. Non-specific binding was defined as the amount of radioactivity remaining bound in the presence of 1μM cold galanin. In competition studies various concentrations of peptides (hGal, pGal, hGal(1-16), rGAL(2-29), rGAL(3-29), hGal (1-19) or chimeric peptides (C7, M15, M40, M35) were included along with ^{125}I-hGal (70 pmol). Incubations were terminated by rapid filtration through GF/C filters which had been presoaked with 0.1% polyethylamine using a TOMTEC (Orange, CT) cell harvester. The results were analyzed using the Prism software package (GraphPad, San Diego, CA). Shown in the table below is the ligand binding profiles of both rat GALR1 and rat GALR2 proteins (clone 27A shown; clone 16.6 gave similar results). The K_D for binding of ^{125}I-labeled human galanin against rat GALR2 was 0.2 nM.

	IC50 (nM)	
	rat GALR1	rat GALR2 (clone 27A)
pig Galanin	0.06	0.46
human Galanin	0.07 ±0.01	1.3 ±0.5
rat Gal (2-29)	7.2	2.9 ±1.3
rat Gal (3-29)	>1000	>1000
human Gal (1-19)	0.86	
pig Gal (1-16)	0.27 ±0.18	3.0
galantide(M15)	1.0 ±1.1	28 ±3.5
C7	4.9 ±3	23 ±13
M40	0.01	1.9 ±0.14
M35	0.9±0.6	0.43 ±0.18

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EXAMPLE 5

Expression of rat GALR2

In situ hybidization was conducted to map the distribution of GALR2 mRNA in rat brain using a ^{32}P-labeled GALR2 ORF fragment as a hybridization probe; see O'Dowd, B. F. et al. 1995 Genomics 28:84-91. Specific hybridization was detected in a number of brain nuclei and regions, most notably supra-, pre-(PMD/ PMV), med- and lateral mammillary nuclei, the dendate gyrus (DG), cingulate gyrus (CG), posterior hypothalamic (PH), supraoptic and arcuate nuclei (Arc) as shown in Figure 16. Both frontal and parietal cortical regions were also labeled.

Clone Isolation of Human GALR2; Cloning of Partial GalR2 gene by degenerate PCR.

Human genomic DNA was amplified by PCR using degenerate oligonucleotides designed based on the sequences encoding transmembranes (TM) regions TM3 (P1: 5' CTG ACC GYC ATG RSC ATT GAC SGC TAC, SEQ ID NO:14, wherein Y = C or T, R=A or G, S = C or G) and TM7 (P2: 5'-GGG GTT GRS GCA GCT GTT GGC RTA, SEQ ID NO:15) of somatostatin receptors and the receptor encoded by the somatostatin-related gene, SLC-1. The PCR conditions were as follows: denaturation at 95°C for 1 min, annealing at either 55°C, 45°C, or 38 °C for 1 min and extension at 72°C for 2.5 min for 30 cycles, followed by a 7 min extension at 72°C. The resultant PCR products were phenol/chloroform extracted, precipitated with ethanol, phosphorylated with T4 polynucleotide kinase, and blunt-ended with Klenow enzyme. Subsequently, they were electrophoresed on a 0.5% low-melting point agarose and a fragment of the expected size was subcloned into the EcoRV site of pBluescript SK(-) (Stratagene, La Jolla, CA). Colonies were selected, plasmid DNA was purified, and the inserts sequenced.

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EXAMPLE 6

Gene Sequence and Structure; Cloning and sequencing of Human GalR2 Genomic DNA.

DNA fragments radiolabelled with [32P]dCTP by nick translation (Amersham) were used as a probe to screen a EMBL3 SP6/T7 human genomic library (Clontech, Palo Alto, CA). Positive phage clones were plaque purified, DNA was prepared, restriction enzyme digested, electrophoresed on an agarose gel, transferred to nylon membrane, and hybridized with the same probe used to screen the library, as described by Marchese et al, 1994 [Genomics 23, 609-618]. Positive phage were subcloned by digesting phage DNA, and subcloning the resultant fragment into the pBluescript vector. The DNA sequence of the clone was determined using standard methods on an ABI 372 automated sequencer (Perkin-Elmer-Applied Biosystems, Foster City, CA). As shown in FIGURE 7, the sequence determined shows a gene with a total of two exons interrupted by an 1800 bp intron. The deduced amino acid sequence (FIGURE 9) of the complete open reading frame (FIGURE 8) gives a protein of 387 amino acids with features typical of G protein-coupled receptors including 7 transmembrane alpha helical domains. Figure 14 shows an alignment of GALR1 and GALR2 protein sequences with the seven transmenbrane domains underlined. The human GALR2 protein bears strong sequence identity and similarity to the rat GALR2 ortholog. One notable difference between the human and rat forms is the presence of an additional 15 amino acids in the C-terminal intracellular domain of human GALR2.

EXAMPLE 7

Receptor Expression: Human and Rat GALR2; Construction of Human GalR2 Expression Plasmid

The human GalR2 expression construct was assembled from the human genomic clone by PCR. Each exon was PCR amplified using standard conditions. The primers for exon I were: Forward, Exon

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I (5' - CCG GAA TTC GGT ACC ATG AAC GTC TCG GGC TGC CC - 3'; SEQ ID NO:16) and Reverse, Exon I (5' - GGT AGC GGA TGG CCA GAT ACC TGT CTA GAG AGA CGG CGG CC - 3'; SEQ ID NO:17). The primers for exon II were: Forward, Exon II (5' - GGC CGC CGT CTC TCT AGA CAG GTA TCT GGC CAT CCG CTA CC - 3'; SEQ ID NO:18) and Reverse, Exon II (5' - GGC CGC CGT CTC TCT AGA CAG GTA TCT GGC CAT CCG CTA CC - 3'; SEQ ID NO:19). PCR products were subcloned in to pBluescript and sequenced. Exon I product was subcloned into the EcoRI and XbaI sites of plasmid pCINeo (Promega, Madison, WI). Exon II was then cloned into the XbaI site and the orientation determined by appropriate restriction digests and DNA sequencing.

EXAMPLE 8

Radioligand binding assay

Plasmid DNA was prepared using the Qiagen Maxi protocol (Qiagen, Chatsworth, CA) and transfected into COS-7 cells by electroporation. Briefly, 0.85 µl COS-7 cells in Ringers' buffer (1.2 x 10^7/ml) and 20 µg of DNA were mixed in a 0.4 mm electroporation cuvette (Bio-Rad, Hercules, Ca) and current (960 µF, 260 V) was applied using a Bio-Rad Electroporator device. Cells were transferred to a T-180 flask (Corning) with fresh media and expression was allowed to proceed for 72 hrs. Membranes were prepared from transfected cells following disruption in enzyme-free dissociation solution (Specialty Media, Lavallette, NJ) in a Dounce homogenizer in ice-cold membrane buffer (10 mM Tris, pH 7.4, 10 mM PMSF, 10 µM phosphoramidon, and 40 µg/ml bacitracin). After a low speed (1100 x g, 10 min. at 4°C) and a high speed centrifugation (38,700 x g for 15 min. at 4°C), membranes were suspended in buffer and the protein concentration determined (Bio-Rad assay kit). Binding of ^{125}I-human galanin (sp. act = 2200 Ci/mmol, DuPont NEN) was measured in membranes using a buffer of 25 mM Tris pH 7.4, 0.5% BSA, 2 mM $MgCl_2$, 40 µg/ml bacitracin, 4 µg/ml phosphoramidon, and 10 µM leupeptin in a total volume of 0.25 ml. 70 pm ^{125}I-human galanin was used. Reactions were initiated by the

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addition of membranes and the incubation was allowed to proceed at room temperature for 1 hour. Non-specific binding was defined as the amount of membrane bound radioactivity remaining in the presence of 1μM cold galanin. In competition studies various concentrations of peptides (hGal, pGal, hGal(1-16), rGAL(2-29), rGAL(3-29), hGal (1-19) or chimeric peptides (C7, M15, M40, M35) were included along with ^{125}I-hGal (70 pmol). Incubations were terminated by rapid filtration through GF/C filters which had been presoaked with 0.1% polyethylamine using a TOMTEC (Orange, CT) cell harvester. The results were analyzed using the Prism software package (GraphPad, San Diego, CA).

Recombinant expression of human GALR2 binding sites in transiently transfected COS-7 permitted the determation of pharmacology of the cloned receptor. ^{125}I-human galanin bound to the cloned GALR2 receptor with high affinity in a saturable and specific manner with a K_D of 5 nM. As summarized in Figure 10, competition of ^{125}I-human galanin with a variety of galanin-derived peptides and chimeric peptide antagonist/partial agonists showed that the human GALR2 receptor has a similar pharmacology of binding to that of the rat GALR2.

EXAMPLE 9

Functional Characterization: Post-receptor signalling mechanism Frog melanophore assay

Growth of *Xenopus laevis* melanophores and fibroblasts was performed as described previously (Potenza, M.N. et al, 1992, *Pigment Cell Res.* 3:38-43). Briefly, melanophores were grown in fibroblast-conditioned growth medium. The fibroblast-conditioned growth medium was prepared by growing fibroblasts in 70% L-15 medium (Sigma), pH 7.3, supplemented with 20% heat-inactivated fetal bovine serum (Gibco), 100 μg/ml streptomycin, 100 units/ml penicillin and 2 mM glutamine at 27.5°C. The medium from growing fibroblasts was collected, passed through a 0.2 μm filter (fibroblast-conditioned growth medium) and used to culture melanophores at 27.5°C.

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Plasmid DNA was transiently transfected into melanophores by electroporation using a BTX ECM600 electroporator (Genetronics, Inc. San Diego, CA). Melanophores were incubated in the presence of fresh fibroblast-conditioned frog medium for 1 hour prior to harvesting of cells. Melanophore monolayers were detached by trypsinization (0.25% trypsin, JHR Biosciences), followed by inactivation of the trypsin with fibroblast-conditioned frog medium. The cells were collected by centrifugation at 200 x *g* for 5 minutes at 4°C. Cells were washed once in fibroblast conditioned frog medium, centrifuged again and resuspended at 5 x 10^6 cells per ml in ice cold 70% PBS pH 7.0. 400 µl aliquots of cells in PBS were added to prechilled eppendorf tubes containing 2 µg of pcIneo:human Galanin 2 receptor plasmid DNA mixed with control receptor cDNA and naked vector DNA for a total of 20 µg DNA (2 µg each of pcDNA1amp:cannabinoid 2 and pcDNA3: thromboxane A2 receptor plasmid DNA, and 18 µg of pcDNA3.1 plasmid DNA in 40 µl total volume, or 2 µg each of pcDNA1amp: cannabinoid 2 and pcDNA3:thromboxane A2 receptor plasmid DNA, and 20 µg of pcDNA3.1 plasmid DNA in 40 µl total volume, as a control). Samples were incubated on ice for 20 min, and mixed every 7 minutes. Cell and DNA mixes were transferred to prechilled 2 mM gap electroporation cuvettes (BTX) and electroporated with the following settings: capacitance of 325 microfarad, voltage of 450 volts and resistance of 720 ohms. Immediately following electroporation, cells were mixed with fibroblast-conditioned frog medium (7.85 mls per cuvette) and plated onto flat bottom 96 well microtiter plates (NUNC). Electroporations from multiple cuvettes were pooled together prior to plating to ensure homogenous transfection efficiency. On the day following transfection, medium was removed and fresh fibroblast-conditioned frog medium was added to the melanophore monolayer and cell were incubated at 27°C.

Cells were assayed for receptor expression 2 days following transfection in 96-well plate format. On the day of ligand stimulation, medium was removed by aspiration and cells were washed with 70% L-15 containing 15 mM HEPES pH 7.3 (Sigma). Assays were dividing into two separate parts in order to examine Gs/Gq functional coupling which results in pigment dispersion in melanophores, or Gi functional coupling which results in pigment aggregation. For Gs/Gq functional coupling responses, assays were performed as follows. Cells were

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incubated in 100 µl of 70% L-15 containing 15 mM HEPES for 1 hour in the dark at room temperature, and then incubated in the presence of melatonin (2 nM final concentration) for 1 hour in the dark at room temperature to induce pigment aggregation. Initial absorbance at 600 nM was measured using a Bio-Tek Elx800 Microplate reader (ESBE Scientific) prior to addition of ligand. Human galanin (Peninsula) was added in duplicate wells, samples were mixed and incubated in the dark at room temperature for 1 hour, after which the final absorbance at 600 nm was determined. For Gi coupled responses, cell monolayers were incubated in the presence of 100 µl of 70% L-15 containing 2% fibroblast-conditioned growth medium, 2 mM glutamine, 100 ug/ml streptomycin, 100 units/ml penicillin and 15 mM HEPES for 15 minutes in the dark at room temperature to preset the cells to dispersion. After initial absorbance at 600 nM was determined, human galanin was added to cell monolayers, samples were mixed, incubated in the dark for 1.5 hour at room temperature and then final absorbances were determined. Absorbance readings were converted to transmission values in order to quantitate pigment dispersion using the following formula: 1- Tf/Ti, where Ti = the initial transmission at 600 nm and Tf = the final transmission at 600 nm. Pigment aggregation was quantitated using the following formula: Af/Ai -1, where Af = final absorbance at 600 nm and Ai is initial absorbance at 600 nm.

To determine whether the human GALR2 could be functionally expressed in melanophores, the expression plasmid pcIneo:hGALR2 was transiently transfected by electroporation into melanophores followed by stimulation of the transfected cells with human galanin. Increasing doses of galanin resulted in a dose-dependent dispersion of pigment in human GALR2-transfected melanophores, in contrast to control vector-transfected cells (FIGURE 11). The apparent EC_{50} for human galanin in pcIneo:hGALR2-transfected melanophores was 20 nM, in general agreement with specific ^{125}human galanin binding in pcIneo:hGALR2-transfected COS-7 cells (IC_{50} ~ 4 nM). The dispersion of pigment in the melanophore has been previously shown to occur either through Gαs coupling and stimulation of adenylyl cyclase or through Gαq coupling and mobilization of calcium.

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There was no detectable aggregation of the pigment in either the pcIneo:hGALR2- or mock-transfected melanophores following incubation in the presence of 0.001 - 1000 nM human galanin. This result suggests that the hGALR2 does not couple to Gαi-mediated signaling pathways.

EXAMPLE 10

Aequorin bioluminescence assay

Measurement of GALR2 expression in the aequorin-expressing stable reporter cell line 293-AEQ17 (Button, D et al, 1993 "Aequorin-expressing mammalian cell lines used to report Ca^{2+} mobilization" *Cell Calcium* 14:663-671) was performed using a Luminoskan RT luminometer (Labsystems Inc., Gaithersburg, MD) controlled by custom software written for a Macintosh PowerPC 6100. 293-AEQ17 cells (8 x 10^5 cells plated 18 hrs. before transfection in a T75 flask) were transfected with 22 µg of rat or human GALR2 plasmid DNA: 264 µg lipofectamine. Following approximately 40 hours of expression the apo-aequorin in the cells was charged for 4 hours with coelenterazine (10 µM) under reducing conditions (300 µM reduced glutathione) in ECB buffer (140 mM NaCl, 20 mM KCl, 20 mM HEPES-NaOH [pH=7.4], 5 mM glucose, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 0.1 mg/ml bovine serum albumin). The cells were harvested, washed once in ECB medium and resuspended to 500,000 cells/ml. 100 µl of cell suspension (corresponding to 5x10^4 cells) was then injected into the test plate, and the integrated light emission was recorded over 30 seconds, in 0.5 second units. 20 mL of lysis buffer (0.1% final Triton X-100 concentration) was then injected and the integrated light emission recorded over 10 seconds, in 0.5 second units. The "fractional response" values for each well were calculated by taking the ratio of the integrated response to the initial challenge to the total integrated luminescence including the Triton-X100 lysis response.

The aequorin bioluminescence assay is a reliable test for identifying G protein-coupled receptors which couple through the Ga protein subunit family consisting of Gq and G11 which leads to the

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activation of phospholipase C, mobilization of intracellular calcium and activation of protein kinase C. Based on the above melanophore data for GALR2, utilization of the aequorin bioluminescence assay permitted the discrimination between the two possibilities for the primary intracellular signaling mechanism for GALR2, namely Gαs coupling and stimulation of adenylyl cyclase or Gαq coupling and mobilization of calcium. Expression of human or rat GALR2 in the aequorin-expressing 293 cell line (293-AEQ17) gave a dose-dependant increase in aequorin bioluminescence in response to challenge by galanin and several related peptides. Transfection of human GALR1, which signals through Gi and the inhibition of adenylyl cyclase, gave no galanin-dependant increase in aequorin bioluminescence. Responses observed for human or rat GALR2 activation were saturable and the rank order of potency was similar to that observed for competition studies for ^{125}I-human galanin binding. EC_{50}'s, given in nM for the human GALR2 (results were similar for the rat GALR2 ortholog) were: human galanin, 32; rat galanin,12; rat galanin (2-29), 31; rat galanin (3-29) >10,000; M35, 44; M40, 8.8. Of interest to note is that the galanin chimeric peptide antagonists (M35 and M40), thought by some to be pure antagonists on the GALR1 receptor, appear to be partial agonists on the GALR2 receptor. These data indicate that the primary signaling mechanism for GALR2 is through the phopholipase C/protein kinase C pathway, in contrast to GALR1, which communicates its intracellular signal by inhibition of adenylyl cyclase through Gi. In addition, while binding and activation of the rat and human GALR2 receptor by galanin is of high affinity and potency, rat or human GALR1 binds and is activated by galanin at a 10-30 fold lower concentration. This observation points to the existence of other undiscovered naturally-occurring ligand systems that may be agonists at the GALR2 receptor.

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EXAMPLE 11

RNA Expression profile of Human GalR2

Northern blotting analysis was utilized to assess the tissue specificity of human GALR2 mRNA expression. As shown in FIGURE 15, modest expression (indicated by one "+") is seen in a variey of brain regions and peripheral tissues, as observed for the rat ortholog of GALR2. The most prevalent transcript size is ~2.2 kb with a band of ~1.5 kb observed in spleen, thymus and prostate. Tissues with significantly higher expression levels (indicated by two or three "+") were placenta, thymus and prostate.

EXAMPLE 12

Chromosome Localization of Human GalR2 Gene

Fluorescence *in situ* hybridization (FISH) of metaphase spread chromosomes derived from human lymphocytes together with DAPI banding patterns was used to map hGalR2 to its chromosome, as described (Heng, H. H. Q. and Tsui, L.-C. *Modes of DAPI banding and simultaneous in situ hybridization*. Chromosoma 102:325-332). FISH data localize the receptor gene to human chromosome 17q25.

EXAMPLE 13

Mouse GALR2: Clone Isolation; Cloning of Mouse GalR2 Genomic Clone

DNA fragments from the Human GalR2 gene were radiolabelled with [32P]dCTP by random octomer labeling (Gibco BRL) and used as a probe to screen a mouse 129sv genomic library (Stratagene). Positive phage clones were plaque purified, DNA was prepared, restriction enzyme digested, electrophoresed on an agarose gel, transferred to nylon membrane, and hybridized with the same probe used to screen the library. A positive NotI fragment was subcloned into pBluescript (Stratagene).

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EXAMPLE 14

Gene Sequence and Structure

DNA sequence encoding the complete ORF for mouse GALR2 (SEQ ID NO:12) is shown in Figure 12. A single intron of 1060 bp divides the ORF into two exons. Removal of the intron allows for conceptual translation to give the predicted GALR2 polypeptide of 371 amino acids (SEQ ID NO:13) as shown in Fig. 13. Compared to both the human and rat orthologs, the mouse protein sequence bears strong identity (85 % and 96 % respectively).

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WHAT IS CLAIMED:

1. Galanin receptor 2 (GALR2), substantially free from associated proteins, or a GALR2-like receptor, wherein the GALR2-like receptor shares at least about 40% homology to GALR2 and has substantially the same biological activity.

2. A GALR2-like receptor according to Claim 1, which shares at least about 50% homology to a GALR2.

3. A GALR2-like receptor according to Claim 1, which shares at least about 75% homology to a GALR2.

4. A GALR2-like receptor according to Claim 1, which shares at least about 85% homology to a GALR2.

5 Rat GALR2 in accordance with Claim 1.

6. GALR2 according to Claim 1 which is SEQ ID NO:5.

7. GALR2 according to Claim 1 which has the sequence of Clone 16.6.

8. A nucleic acid, substantially free from associated nucleic acids, which encodes a GALR2 or a GALR2-like receptor which is at least about 40 % homologous to GALR2 and which has substantially the same biological activity.

9. A nucleic acid encoding a GALR2-like receptor according to Claim 8, wherein the GALR2-like receptor shares at least about 50% homology to a GALR2.

10. A nucleic acid encoding a GALR2-like receptor according to Claim 8, wherein the GALR2-like receptor shares at least about 75% homology to a GALR2.

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11. A nucleic acid encoding a GALR2-like receptor according to Claim 8, wherein the GALR2-like receptor shares at least about 85% homology to human GALR2.

12. A nucleic acid according to Claim 8 which is DNA.

13. A vector comprising the nucleic acid of Claim 8.

14. A host cell comprising the nucleic acid of Claim 8.

15. A method of determining if a compound is a GALR2 ligand comprising contacting the compound and GALR2 and determining if binding occurs.

16. A method of identifying a compound that modulates GALR2 receptor activity, comprising:

(a) culturing cells expressing GALR2 receptor in the presence of the compound; and

(b) measuring GALR2 receptor activity or second messenger activity.

17. A method according to Claim 16 wherein the cells are transformed to express a GALR2 receptor.

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GCGTGCTCAC CAAGACCCGG ACAGCTGCGG GAGCGGCGTC 280
CACTTTGGTG ATACCATGAA TGGCTCCGGC AGCCAGGGCG 320
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TGCTGCTGCG CGGCGGCCAG GCGGTCAGCA CCACCAACCT 480
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CTGTGCTGCG TGCCTTTCCA GGCCACCATC TACACCCTGG 560
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FIG. 1A

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FIG. 1B

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FIG. 2A

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FIG. 2B

BNSDOCID <WO 9829439A1 : >

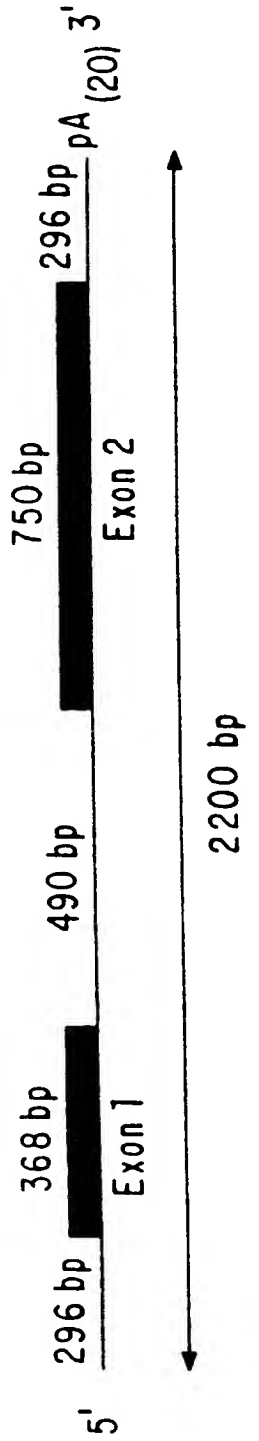

FIG. 3

BNSDOCID <WO 9829439A1 | >

SUBSTITUTE SHEET (RULE 26)

BNSDOCID <WO 9829439A1 >

```
   1  ATG AAT GGC TCC GGC AGC CAG GGC GCG GAG AAC ACG AGC CAG GAA GGC GGT AGC GGC GGC TGG CAG CCT GAG GCG   75
   1  M   N   G   S   G   S   Q   G   A   E   N   T   S   Q   E   G   G   S   G   G   W   Q   P   E   A   25

  76  GTC CTT GTA CCC CTA TTT TTC GCG CTC ATC TTC CTC GTG GGC ACC GTG GGC AAC GCG CTG GTG CTG GCG GTG CTG  150
  26  V   L   V   P   L   F   F   A   L   I   F   L   V   G   T   V   G   N   A   L   V   L   A   V   L   50

 151  CTG CGC GGC GGC CAG GCG GTC AGC ACC ACC AAC CTG TTC ATC CTC AAC CTG GGC GTG GCC GAC CTG TGT TTC ATC  225
  51  L   R   G   G   Q   A   V   S   T   T   N   L   F   I   L   N   L   G   V   A   D   L   C   F   I   75

 226  CTG TGC TGC GTG CCT TTC CAG GCC ACC ATC TAC ACC CTG GAC GAC TGG GTG TTC GGC TCG CTG CTC TGC AAG GCT  300
  76  L   C   C   V   P   F   Q   A   T   I   Y   T   L   D   D   W   V   F   G   S   L   L   C   K   A  100

 301  GTT CAT TTC CTC ATC TTT CTC ACT ATG CAC GCC AGC AGC TTC ACG CTG GCC GCC GTC TCC CTG GAC AG          368
 101  V   H   F   L   I   F   L   T   M   H   A   S   S   F   T   L   A   A   V   S   L   D   R       123

 369  gtaaaggacccagaaagaaacatccagtatgcccggagggatcttgactggaaaagactgaatcctggtctggtgacctt
 449  agttccctgccctttcacatcacttggacattcccacagaagagcggtgaagaggcggtggtccttattctcctctggtt
 529  tccactgagtgcaacatgtgcgtcctgagtacgctggagggactcacaaaatttcagctttctttaggagtttccttgct
 609  gtagtttgacccaagtcttctccaggtttctgtcagaacctcaggcatgagggatctgcctcccctggttgtcaccagag
 689  gataacaatcactgcccccagaaatccagacagattctacaactttagtcttcggtgttttgggggtgccccttcacgt
 769  ggagtaggtcggtggccacattcccaggagtgacaatagcctagcagtgaatcctctcgcttagctgatgccccccact
 849  gtccccacag

 859  G TAT CTG GCC ATC CGC TAC CCG CTG CAC TCC CGA GAG TTG CGC ACA CCT CGA AAC GCG CTG GCC GCC ATC GGG      931
 124    Y   L   A   I   R   Y   P   L   H   S   R   E   L   R   T   P   R   N   A   L   A   A   I   G  147

 932  CTC ATC TGG GGG CTA GCA CTG CTC TTC TCC GGG CCC TAC CTG AGC TAC TAC CGT CAG TCG CAG CTG GCC AAC CTG  1006
 148  L   I   W   G   L   A   L   L   F   S   G   P   Y   L   S   Y   Y   R   Q   S   Q   L   A   N   L   50

1007  ACA GTA TGC CAC CCA GCA TGG AGC GCA CCT CGA CGT CGA GCC ATG GAC CTC TGC ACC TTC GTC TTT AGC TAC CTG  1081
 151  T   V   C   H   P   A   W   S   A   P   R   R   R   A   M   D   L   C   T   F   V   F   S   Y   L  172

1082  CTG CCA GTG CTA GTC CTC AGT CTG ACC TAT GCG CGT ACC CTG CGC TAC CTC TGG CGC ACA GTC GAC CCG GTG ACT  1156
 173  L   P   V   L   V   L   S   L   T   Y   A   R   T   L   R   Y   L   W   R   T   V   D   P   V   T  222
```

FIG.3A

SUBSTITUTE SHEET (RULE 26)

BNSDOCID <WO 9829439A1 >

```
1157  GCA GGC TCA GGT TCC CAG CGC GCC AAA CGC AAG GTG ACA CGG ATG ATC ATC ATC GTG GCG GTG CTT TTC TGC CTC  1231
 223  A   G   S   G   S   Q   R   A   K   R   K   V   T   R   M   I   I   I   V   A   V   L   F   C   L    247

1232  TGT TGG ATG CCC CAC CAC GCG CTT ATC CTC TGC GTG TGG TTT GGT CGC TTC CCG CTC ACG CGT GCC ACT TAC GCG  1306
 248  C   W   M   P   H   H   A   L   I   L   C   V   W   F   G   R   F   P   L   T   R   A   T   Y   A    272

1307  TTG CGC ATC CTT TCA CAC CTA GTT TCC TAT GCC AAC TCC TGT GTC AAC CCC ATC GTT TAC GCT CTG GTC TCC AAG  1381
 273  L   R   I   L   S   H   L   V   S   Y   A   N   S   C   V   N   P   I   V   Y   A   L   V   S   K    297

1382  CAT TTC CGT AAA GGT TTC CGC AAA ATC TGC GCG GGC CTG CTG CGC CCT GCC CCG AGG CGA GCT TCG GGC CGA GTG  1456
 298  H   F   R   K   G   F   R   K   I   C   A   G   L   L   R   P   A   P   R   R   A   S   G   R   V    322

1457  AGC ATC CTG GCG CCT GGG AAC CAT AGT GGC AGC ATG CTG GAA CAG GAA TCC ACA GAC CTG ACA CAG GTG AGC GAG  1531
 323  S   I   L   A   P   G   N   H   S   G   S   M   L   E   Q   E   S   T   D   L   T   Q   V   S   E    347

1532  GCA GCC GGG CCC CTT GTC CCA CCA CCC GCA CTT CCC AAC TGC ACA GCC TCG AGT AGA ACC CTG GAT CCG GCT TGT  1606
 348  A   A   G   P   L   V   P   P   P   A   L   P   N   C   T   A   S   S   R   T   L   D   P   A   C    372

1607  TAA                                                                                                  1609
      *
```

FIG.3B

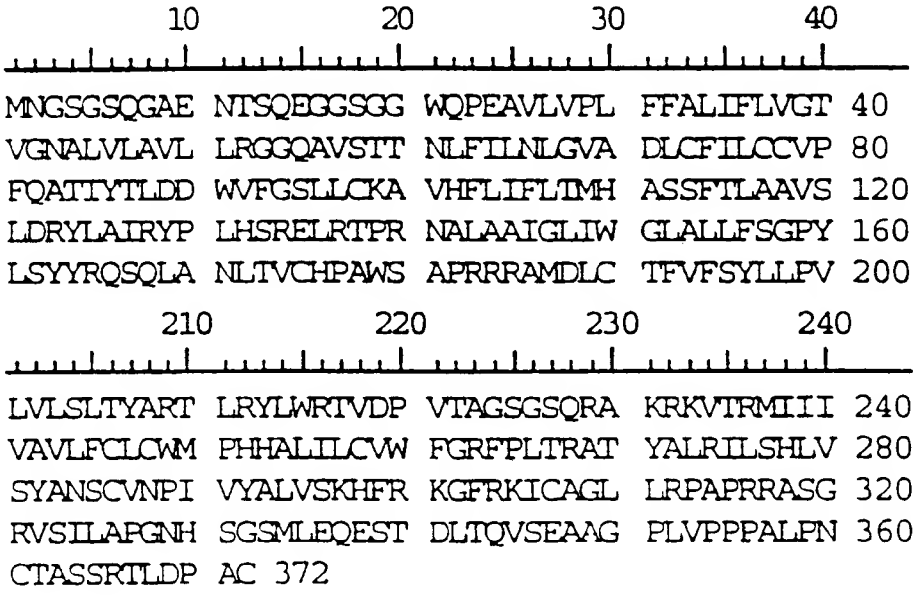

FIG. 4

BNSDOCID <WO 9829439A1 >

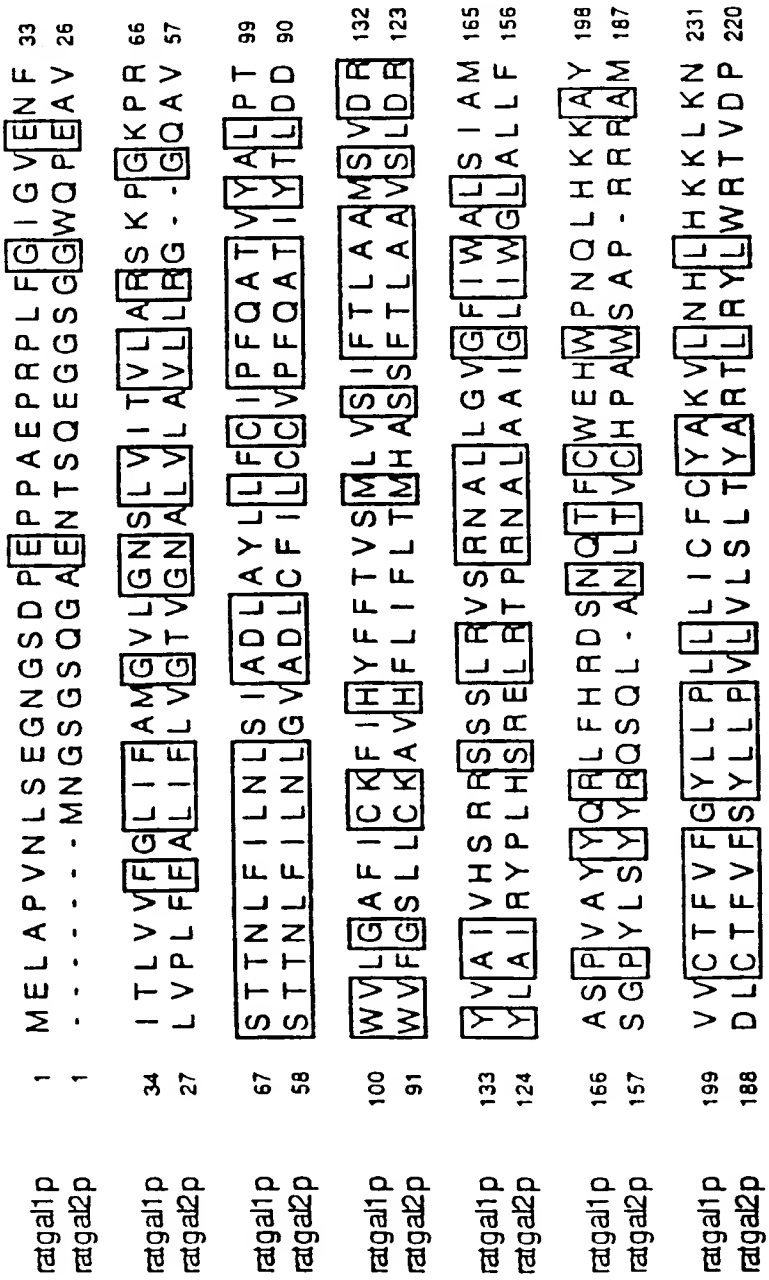

FIG. 5A

SNSDOCID <WO 9829439A1 >

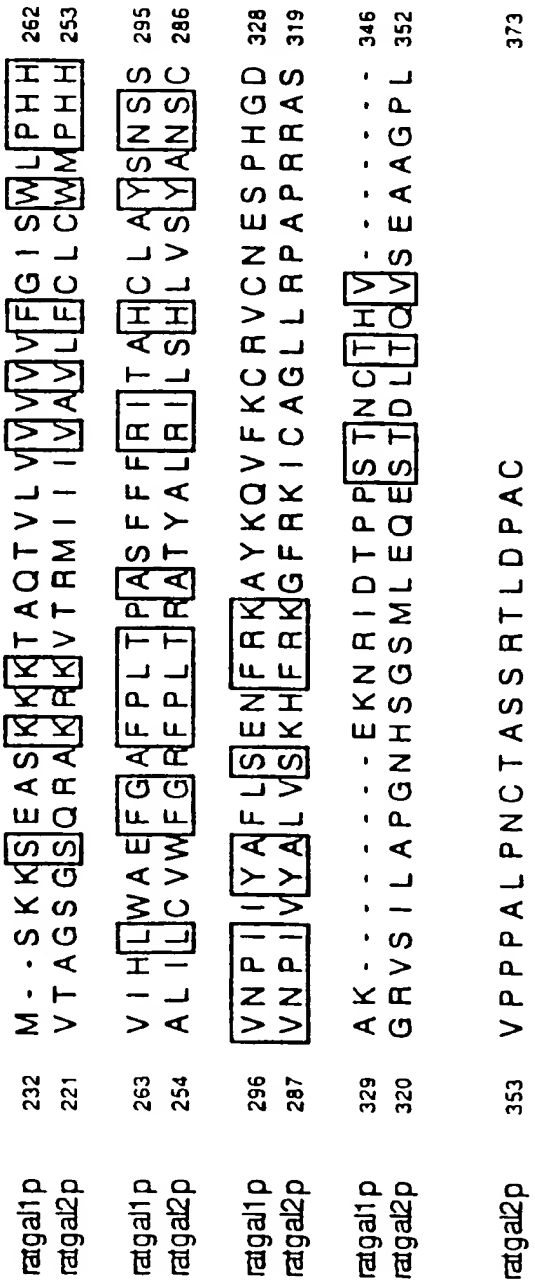

FIG. 5B

BNSDOCID <WO 9829439A1 >

1
TGCGGACCACCACCAACTTGTACCTGGGCA 30

GCATGGCCGTGTCCGACCTACTCATCCTGC 60

TCGGGCTGCCGTTCGACCTGTACCGCCTCT 90

GGCGCTCGCGGCCCTGGGTGTTCGGGCCGC 120

TGCTCTGCCGCCTGTCCCTCTACGTGGGCG 150

AGGGCTGCACCTACGCCACGCTGCTGCACA 180

TGACCGCGCTCAGCGTCGAGCGCTACCTGG 210

CCATCTGCCGCCCGCTCCGCGCCCGCGTCT 240

TGGTCACCCGGCGCCGCGTCCGCGCGCTCA 270

TCGCTGTGCTCTG 283

FIG.6

BNSDOCID <WO 9829439A1 | >

gagctcggaagcaggtacaagcgccactctccgcctgcgccgtggaatgcgcgccgggacc
antccgcagcccttcccccagcgccgccggccgctgctggggacaacctcgccctcctgtn
tcttgctcctcctcctgaccccagcgcacccccatccccgccccagatgaggcaaggctcc
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TGAACGTCTCGGGCTGCCCAGGGGCCGGGAACGCGAGCCAGGCGGGCGGCGGGGGAGGCTG
GCACCCCGAGGCGGTCATCGTGCCCCTGCTCTTCGCGCTCATCTTCCTCGTGGGCACCGTG
GGCAACACGCTGGTGCTGGCGGTGCTGCTGCGCGGCGGCCAGGCGGTCAGCACTACCAACC
TGTTCATCCTTAACCTGGGCGTGGCCGACCTGTGTTTCATCCTGTGCTGCGTGCCCTTCCA
GGCCACCATCTACACCCTGGACGGCTGGGTGTTCGGCTCGCTGCTGTGCAAGGCGGTGCAC
TTCCTCATCTTCCTCACCATGCACGCCAGCAGCTTCACGCTGGCCGCCGTCTCCCTGGACA
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agacaagcgggcgcggaggaggaaaaagaggaataagaatgggggaccgtggtgtccctcg
gttagatgcgtcctggggcctggaagcctggagaatgtggctctccagcgccgcccgtgcc
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caggtggagcttgaaaggacactgggatggtttccctggggaggaaatccgggtatttccc
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cgctaaggaccttccttgagagcagccttgggaccgaggtgcaggggtcgcggccctccag
catgaatgtgcccgctcagccgacgtctcccttcccggtctgaccgcagGTATCTGGCCAT
CCGCTACCCGCTGCACTCCCGCGAGCTGCGCACGCCTCGAAACGCGCTGGCAGCCATCGGG
CTCATCTGGGGGCTGTCGCTGCTCTTCTCCGGGCCCTACCTGAGCTACTACCGCCAGTCGC

FIG.7A

BNSDOCID <WO 9829439A1 >

AGCTGGCCAACCTGACCGTGTGCCATCCCGCGTGGAGCGCCCCTCGCCGCCGCGCCATGGA
CATCTGCACCTTCGTCTTCAGCTACCTGCTTCCTGTGCTGGTTCTCGGCCTGACCTACGCG
CGCACCTTGCGCTACCTCTGGCGCGCCGTCGACCCGGTGGCCGCGGGCTCGGGTGCCCGGC
GCGCCAAGCGCAAGGTGACACGCATGATCCTCATCGTGGCCGCGCTCTTCTGCCTCTGCTG
GATGCCCCACCACGCGCTCATCCTCTGCGTGTGGTTCGGCCAGTTCCCGCTCACGCGCGCC
ACTTATGCGCTTCGCATCCTCTCGCACCTGGTCTCCTACGCCAACTCCTGCGTCAACCCCA
TCGTTTACGCGCTGGTCTCCAAGCACTTCCGCAAAGGCTTCCGCACGATCTGCGCGGGCCT
GCTGGGCCGTGCCCCAGGCCGAGCCTCGGGCCGTGTGTGCGCTGCCGCGCGGGGCACCCAC
AGTGGCAGCGTGTTGGAGCGCGAGTCCAGCGACCTGTTGCACATGAGCGAGGCGGCGGGGG
CCCTTCGTCCCTGCCCCGGCGCTTCCCAGCCATGCATCCTCGAGCCCTGTCCTGGCCCGTC
CTGGCAGGGCCCAAAGGCAGGCGACAGCATCCTGACGGTTGATGTGGCCTGAaagcactta
gcgggcgcgctgggatgtcacagagttggagtcattgttgggggaccgtggggagagcttt
gcctgttaataaaacgcacaaaccatttcacacacagtgacagcgctgtttcgcgtttctc
attgtcttgagattctgggaggaagcctctggggcttcacagaggggctccctaggggtaa
gtgcaggacccttgcagagctaccaggaaagagggctgatcacacctcaggcagccgggt
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ggcgttcgataaagtcggttgatgacagacacagatgtgtgttcccagccgcatttgtgct
ctggtgtgtgacaggtctgtccttgcctgctttcagctcccagggcccctttgagtctggg
cagcccagtcagtccccgtccatttttggccttagcttttccttccctggctacatctggg
ccaggatcaagtctccagcagctgtttcactcccc

FIG.7B

BNSDOCID <WO 9829439A1 >

ATGAACGTCTCGGGCTGCCCAGGGGCCGGGAACGCGAGCCAGGCGGGCGGCGGGGGAGGCT
GGCACCCCGAGGCGGTCATCGTGCCCCTGCTCTTCGCGCTCATCTTCCTCGTGGGCACCGT
GGGCAACACGCTGGTGCTGGCGGTGCTGCTGCGCGGCGGCCAGGCGGTCAGCACTACCAAC
CTGTTCATCCTTAACCTGGGCGTGGCCGACCTGTGTTTCATCCTGTGCTGCGTGCCCTTCC
AGGCCACCATCTACACCCTGGACGGCTGGGTGTTCGGCTCGCTGCTGTGCAAGGCGGTGCA
CTTCCTCATCTTCCTCACCATGCACGCCAGCAGCTTCACGCTGGCCGCCGTCTCCCTGGAC
AGGTATCTGGCCATCCGCTACCCGCTGCACTCCCGCGAGCTGCGCACGCCTCGAAACGCGC
TGGCAGCCATCGGGCTCATCTGGGGGCTGTCGCTGCTCTTCTCCGGGCCCTACCTGAGCTA
CTACCGCCAGTCGCAGCTGGCCAACCTGACCGTGTGCCATCCCGCGTGGAGCGCCCCTCGC
CGCCGCGCCATGGACATCTGCACCTTCGTCTTCAGCTACCTGCTTCCTGTGCTGGTTCTCG
GCCTGACCTACGCGCGCACCTTGCGCTACCTCTGGCGCGCCGTCGACCCGGTGGCCGCGGG
CTCGGGTGCCCGGCGCGCCAAGCGCAAGGTGACACGCATGATCCTCATCGTGGCCGCGCTC
TTCTGCCTCTGCTGGATGCCCCACCACGCGCTCATCCTCTGCGTGTGGTTCGGCCAGTTCC
CGCTCACGCGCGCCACTTATGCGCTTCGCATCCTCTCGCACCTGGTCTCCTACGCCAACTC
CTGCGTCAACCCCATCGTTTACGCGCTGGTCTCCAAGCACTTCCGCAAAGGCTTCCGCACG
ATCTGCGCGGGCCTGCTGGGCCGTGCCCCAGGCCGAGCCTCGGGCCGTGTGTGCGCTGCCG
CGCGGGGCACCCACAGTGGCAGCGTGTTGGAGCGCGAGTCCAGCGACCTGTTGCACATGAG
CGAGGCGGCGGGGGCCCTTCGTCCCTGCCCCGGCGCTTCCCAGCCATGCATCCTCGAGCCC
TGTCCTGGCCCGTCCTGGCAGGGCCCAAAGGCAGGCGACAGCATCCTGACGGTTGATGTGG
CCTGA

FIG.8

BNSDOCID <WO 9829439A1 >

```
gca Ala(A) 2 # cag Gln(Q)   8 # uug Leu(L)  3  # uaa Ter(.)  0
gcc Ala(A) 23 # --- Gln(Q)  8 # --- Leu(L)  56 # uag Ter(.)  0
gcg Ala(A) 19 # gaa Glu(E)  0 # aaa Lys(K)  1  # uga Ter(.)  1
gcu Ala(A)  2 # gag Glu(E)  6 # aag Lys(K)  5  # --- Ter(.)  1
--- Ala(A) 46 # --- Glu(E)  6 # --- Lys(K)  6  # aca Thr(T)  1
aga Arg(R)  0 # gga Gly(G)  1 # aug Met(M)  6  # acc Thr(T) 10
agg Arg(R)  1 # ggc Gly(G) 25 # --- Met(M)  6  # acg Thr(T)  6
cga Arg(R)  2 # ggg Gly(G)  7 # uuc Phe(F) 17  # acu Thr(T)  2
cga Arg(R) 19 # ggu Gly(G)  1 # uuu Phe(F)  0  # --- Thr(T) 19
cgg Arg(R)  2 # --- Gly(G) 34 # --- Phe(F) 17  # ugg Trp(W)  8
cgu Arg(R)  3 # cac His(H) 10 # cca Pro(P)  4  # --- Trp(W)  8
--- Arg(R) 27 # cau His(H)  1 # ccc Pro(P) 10  # uac Tyr(Y) 10
aac Asn(N)  9 # --- His(H) 11 # ccg Pro(P)  4  # uau Tyr(Y)  2
aau Asn(N)  0 # aua Ile(I)  0 # ccu Pro(P)  4  # --- Tyr(Y) 12
--- Asn(N)  9 # auc Ile(I) 18 # --- Pro)P) 22  # gau Val(V)  0
gac Asp(D)  7 # auu Ile(I)  0 # agc Ser(S) 11  # guc Val(V)  9
gau Asp(D)  1 # --- Ile(I) 18 # agu Ser(S)  1  # gug Val(V) 18
--- Asp(D)  8 # cua Leu(L)  0 # uca Ser(S)  0  # guu Val(V)  3
ugc Cys(C) 14 # cuc Leu(L) 17 # ucc Ser(S)  9  # --- Val(V) 30
ugu Cys(C)  2 # cug Leu(L) 32 # ucg Ser(S)  7  # nnn ???(X)  0
--- Cys(C) 16 # cuu Leu(L)  4 # ucu Ser(S)  0  # TOTAL      388
```

FIG.9A

BNSDOCID <WO 9829439A1 >

MNVSGCPGAGNASQAGGGGGWHPEAVIVPLLFALIFLVGTVGNTL
VLAVLLRGGQAVSTTNLFILNLGVADLCFILCCVPFQATIYTLDGWV
FGSLLCKAVHFLIFLTMHASSFTLAAVSLDRYLAIRYPLHSRELRTPR
NALAAIGLIWGLSLLFSGPYLSYYRQSQLANLTVCHPAWSAPRRRA
MDICTFVFSYLLPVLVLGLTYARTLRYLWRAVDPVAAGSGARRAK
RKVTRMILIVAALFCLCWMPHHALILCVWFGQFPLTRATYALRILS
HLVSYANSCVNPIVYALVSKHFRKGFRTICAGLLGRAPGRASGRVC
AAARGTHSGSVLERESSDLLHMSEAAGALRPCPGASQPCILEPCPGP
SWQGPKAGDSILTVDVA

FIG.9B

BNSDOCID <WO 9829439A1 | >

Pharmacology of Human and Rat GALR2 IC_{50} (nM)			
PEPTIDE	hGALR2	rat GALR2	hGALR1*
human galanin	3.8 ± 0.28	1.5 ± 0.45	0.13 ± 0.04
porcine galanin	1.5 ± 0.03	0.83 ± 0.5	0.14 ± 0.04
rat galanin	1.6 ± 0.42	0.9	0.1
rat Gal (2-29)	15.4 ± 7.9	2.9 ± 0.9	17 ± 7.5
rat Gal (3-29)	>1000	>1000	>1000
M40	9.5 ± 0.7	1.8 ± 1.8	0.48 ± 0.2
M35	5.6 ± 0.2	0.43 ± 0.18	0.04 ± 0.02
C7	40.5 ± 19	13.5 ± 0.7	6.3 ± 6.7
Kd	5 nM	0.19 nM	0.07 nM

FIG.10

BNSDOCID <WO 9829439A1 | >

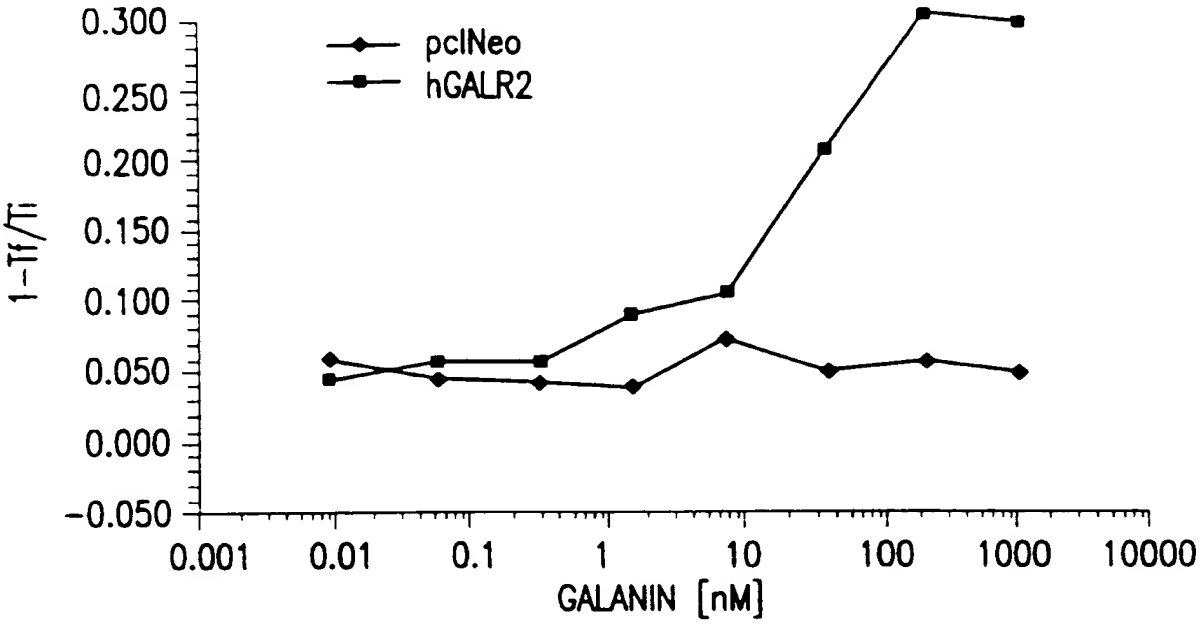

FIG.11A

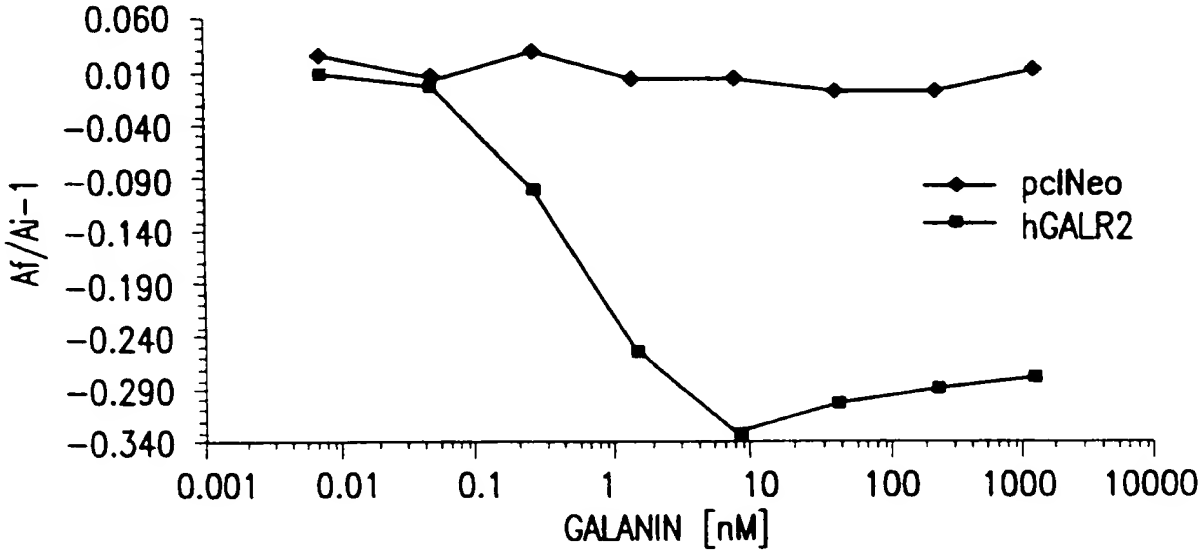

FIG.11B

BNSDOCID <WO_ 9829439A1 >

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GGACTCGAGCCAGGAAGGTGGCGGCGGCTGGCAGCCCGAGGCG
GTCCTCGTACCCCTATTTTTCGCGCTCATCTTCCTCGTGGGCGCTG
TGGGCAACGCGCTGGTGCTGGCGGTGCTGCTGCGCGGCGGCCAG
GCGGTCAGCACCACGAACCTATTCATCCTCAACCTGGGTGTGGC
CGACCTGTGTTTCATCCTGTGCTGCGTGCCTTTCCAGGCCACCATC
TATACCCTGGACGATTGGGTGTTTGGCTCACTGCTCTGCAAGGCC
GTTCATTTCCTCATCTTCCTCACTATGCACGCCAGCAGCTTCACGC
TGGCCGCTGTCTCGCTGGACAGgtgagtgaacattctgtggtgtctgagaactgggt
acccaggtaggagcttgcactggagtcgccacgcaaggatccagaagggatgcgtagtcggggag
aacactaaaattacaaagtggcccgaggccgtgaaacgcaaggggaaaggggactaagactccg
tgactaagagtgtcccttgattaagtcggtcctcagacctcgaaggctggagaaatcggatttctgggg
tctttacgttattgttgcttgagctaaaagtctctcagaaacattgcagtactcagaccagagttggcttg
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CGAAACGCGCTGGCGGCCATCGGGCTCATCTGGGGGCTAGCACT
GCTCTTCTCCGGGCCCTACCTGAGCTACTACAGTCAGTCGCAGCT
GGCCAATCTGACGGTGTGCCACCCAGCGTGGAGCGCACCACGAC
GTCGCGCCATGGACCTCTGCACTTTTGTCTTTAGCTACCTGTTGCC
AGTGCTGGTGCTCAGCCTGACCTATGCGCGCACCCTGCACTACCT
CTGGCGCACAGTTGACCCAGTAGCTGCAGGCTCAGGTTCCCAGC
GCGCCAAGCGCAAGGTGACACGGATGATCGTCATCGTGGCGGTA
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TGTGGTTTGGTCGCTTTCCGCTCACGCGTGCCACTTACGCCCTGC
GCATCCTTTCACATCTAGTATCTTATGCCAACTCGTGTGTCAACCC
CATCGTTTATGCTCTGGTCTCCAAGCATTTCCGCAAAGGTTTCCG
CAAAATCTGCGCGGGCCTGCTACGCCGTGCCCCGAGGAGAGCTT
CAGGCCGAGTGTGCATCCTGGCGCCTGGAAACCATAGTGGTGGC
ATGCTGGAACCTGAGTCCACAGACCTGACACAGGTGAGCGAGG
CAGCCGGGCCCCTCGTCCCCGCACCCGCACTTCCCAACTGCACA
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aacagcttctaagggcga

FIG. 12

BNSDOCID <WO 9829439A1 >

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SLLCKAVHFLIFLTMHASSFTLAAVSLDRYLAIRYPMHSRELRTPRN
ALAAIGLIWGLALLFSGPYLSYYSQSQLANLTVCHPAWSAPRRRAM
DLCTFVFSYLLP VLVLSLTYARTLHYLWRTVDPVAAGSGSQRAKRK
VTRMIVIVAVLF CLCWMPHHALILCVWFGRFPLTRATYALRILSHL
VSYANSCVNPIVYALVSKHFRKGFRKICAGLLRRAPRRASGRVCIL
APGNHSGGMLEPESTDLTQVSEAAGPLVPAPALPNCTTLSRTLDPAC

FIG.13

BNSDOCID <WO 9829439A1 >

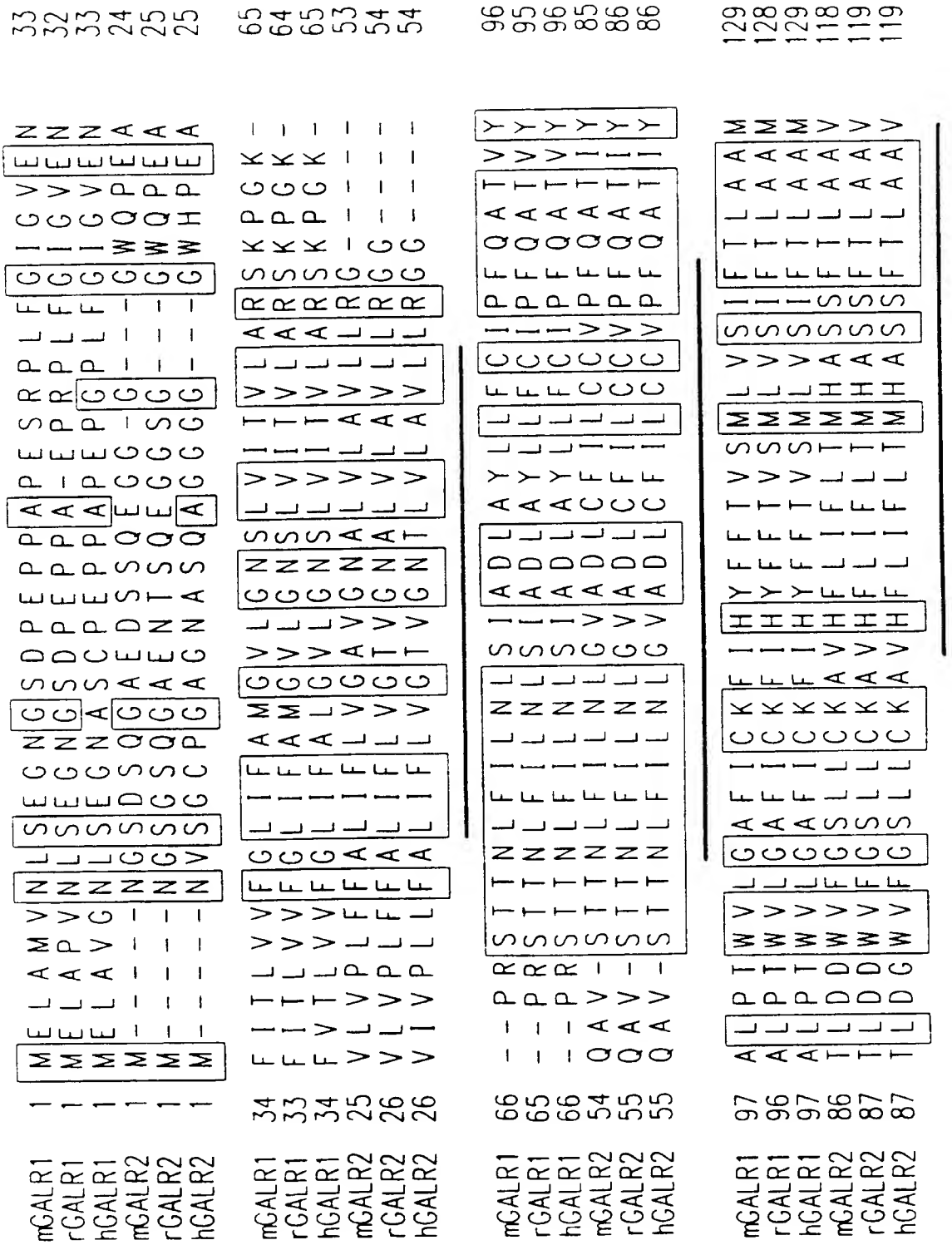

FIG.14A

BNSDOCID <WO 9829439A1 >

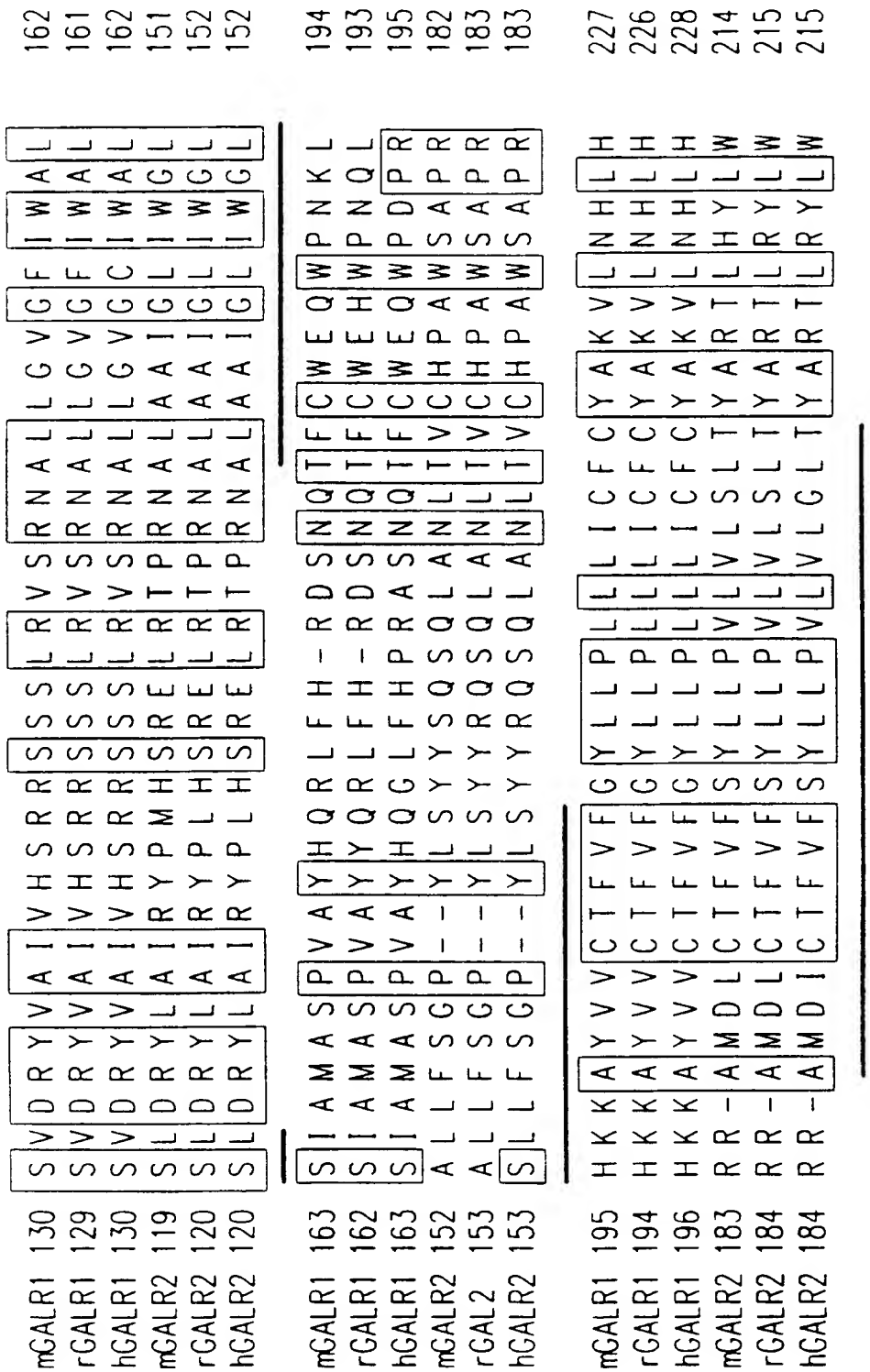

FIG.14B

BNSDOCID <WO 9829439A1 I >

SUBSTITUTE SHEET (RULE 26)

BNSDOCID <WO 9829439A1 >

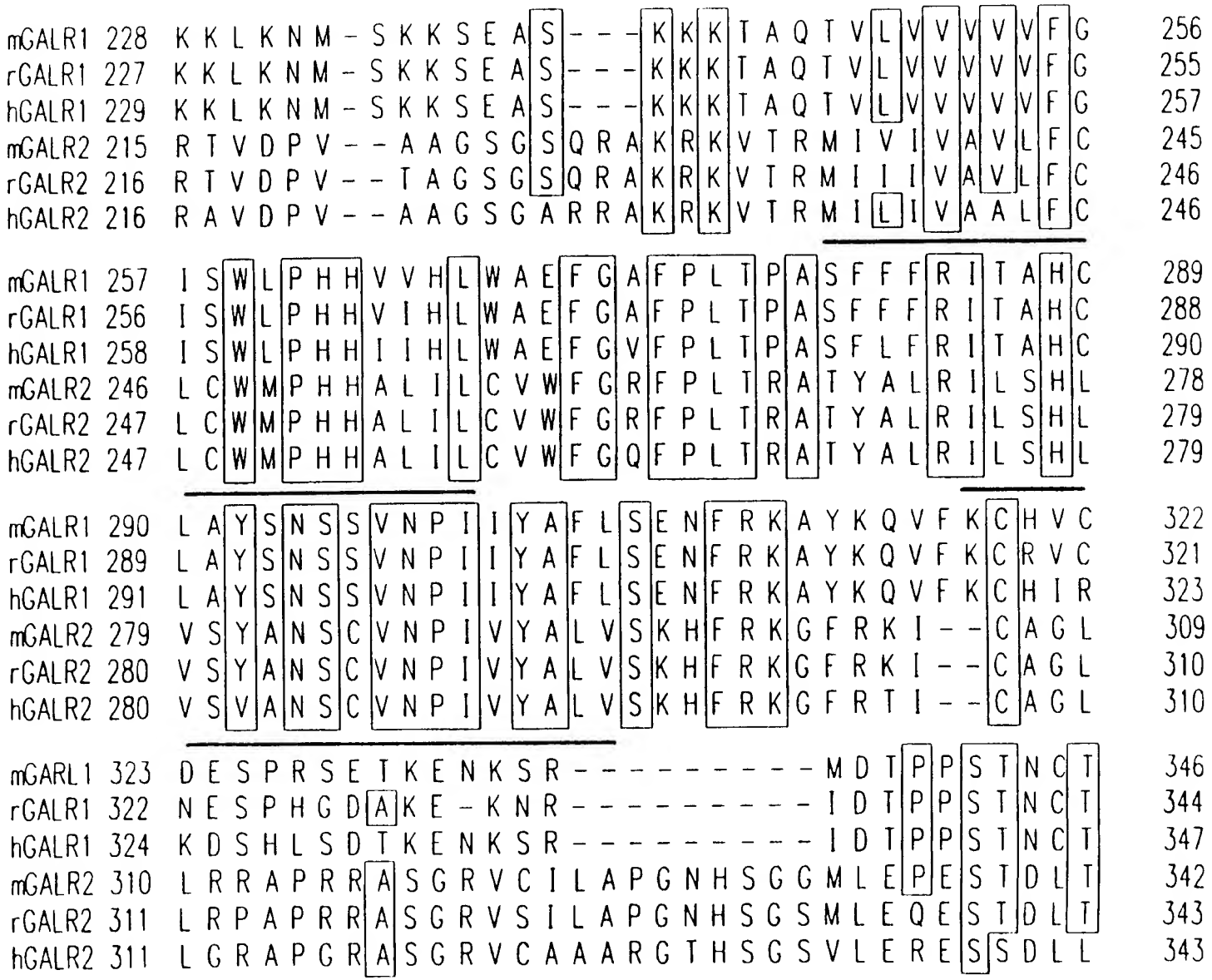

FIG.14C

```
mGALR1 347 HV-------------------------------  348
rGALR1 345 HV-------------------------------  346
hGALR1 348 HV-------------------------------  349
mGALR2 343 QVSEAAGPLVPAPA------LPNCTTLSRTLDP  369
rGALR2 344 QVSEAAGPLVPPPA------LPNCTASSRTLDP  370
hGALR2 344 HMSEAAGALRPCPGASQPCILEPCPGPSWQGPK  376

mGALR1   0 -----------  348
rGALR1   0 -----------  346
hGALR1   0 -----------  349
mGALR2 370 AC---------  371
rGALR2 371 AC---------  372
hGALR2 377 AGDSILTVDVA  387
```

FIG.14D

BNSDOCID <WO 9829439A1 I >

Tissue	Expression Level	Tissue	Expression Level
Total Brain	+	Prostate	+++
Cerebellum	+	Thymus	++
Cerebral Cortex	+	Spleen	+
Medulla	+	Pancreas	+
Occipital Pole	+	Placenta	++
Frontal Pole	+	Heart	-
Temporal Lobe	+	Lung	-
Putamen	+	Liver	-
Spinal Cord	+	Skeletal muscle	-
Amygdala	+	Kidney	-
Caudate Nucleus	+	Testis	-
Corpus Callosum	+	Ovary	-
Hippocampus	+	Small intestine	-
Substantia Nigra	+	Colon	-
Subthalamic n.	+	Blood Leukocyte	-
Thalamus	+		

FIG.15

BNSDOCID <WO 9829439A1 >

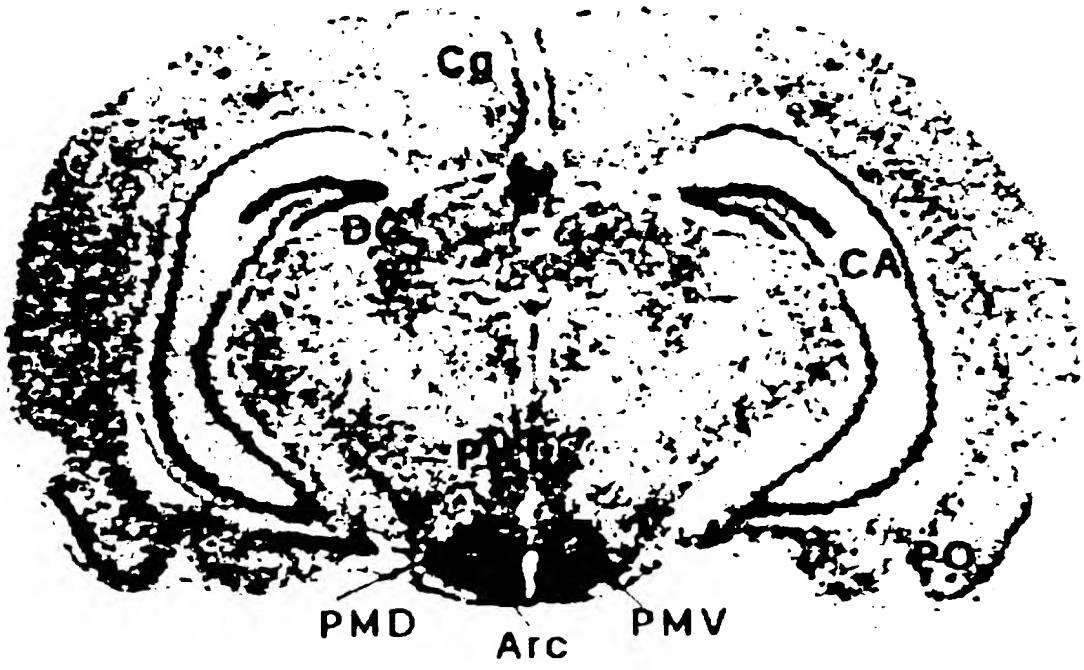

FIG.16

BNSDOCID <WO 9829439A1 >

INTERNATIONAL SEARCH REPORT

International application No
PCT/US97/23890

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :435/7.1, 69.1, 320.1, 325; 530/350; 536/23.5

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.1, 69.1, 320.1, 325; 530/350; 536/23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A, P	WO 97/466681 A2 (BAYER CORPORATION) 11 December 1997, pages 3, and 9-10.	1-17
X	AHMAD et al. Molecular cloning of a novel widely distributed galanin receptor subtype (GALR2). Abstracts: 8th World Congress on Pain. 19 August 1996. Canada: IASP Press. page 134.	15-17
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A		1-14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 24 MARCH 1998	Date of mailing of the international search report 28 APR 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MICHAEL D. PAK Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

BNSDOCID <WO 9829439A1 | >

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/23890

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1 ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2 ☒ Claims Nos.: 1-17 (in part)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Please See Extra Sheet.

3 ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US97/23890

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C07K 14/00, 14/435, 14/705; C12N 5/10, 15/11, 15/63; G01N 33/53, 33/566

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, STN, BIOSCIENCE, BIOSIS, CAPLUS, MEDLINE, SCISEARCH, WPIDS

search terms: galanin?(5a)receptor?, galr#, G-protein?(5a)receptor?, rat

BOX I. OBSERVATIONS WHERE CLAIMS WERE FOUND UNSEARCHABLE
2. Where no meaningful search could be carried out, specifically:

All of the claims are unsearchable to the extent that they require reference to the specified sequences from the sequence listing. Because applicant has not furnished a machine-readable copy of the sequence listing as required by PCT Rule 5.2, no meaningful search of the sequences per se can be carried out by this Authority. However, the subject matter of the claims has been searched to the extent possible with reference to the balance of the description.

BNSDOCID <WO 9829439A1 | >